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N-alkane as an Internal Market for Predicting Digestibility of Forages

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N-alkane as an Internal Marker for Predicting Digestibility of Forages

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N-alkanes may be used as internal markers to predict forage digestibility and may be a suitable alternative to other traditionally used internal markers.

Summary

Independent digestion trials were conducted with three immature grasses, mature grass hay, and alfalfa hay to compare n-alkane with indigestible ADF (IADF) as internal markers to predict in vivo *dry matter digestibility (DMD). Forage DMD estimated with n-alkane ratios were lower than* in vivo *DMD. N-alkanes predicted higher DMD than IADF for alfalfa hay and two of the immature grasses. Comparison of freeze-drying and oven-drying on fecal n-alkane concentrations showed oven-drying reduced amounts of n-alkane extracted for alfalfa hay but had no effect on grass hay. Although fecal recovery of markers was incomplete, more n-alkane was recovered than IADF.*

Introduction

Forage dry matter digestibility (DMD) is an essential tool for assessing the nutrient status of grazing animals. While many different methods have been developed to estimate DMD, errors present due to variation in dry matter intake, physical form of the forage, and age and species of the animals can be corrected with an internal marker (indigestible plant component recoverable in the feces). Several internal markers, such as acid insoluble ash, lignin and indigestible detergent fibers, have been investigated for their potential to estimate digestibility; however, none of these markers exhibit the characteristics of an ideal internal marker.

In recent years, it has been proposed long chain hydrocarbons (n-alkanes) found in plant cuticular wax may be utilized as internal markers. The n-alkanes found in most pasture species have odd-numbered carbon chains containing 25-35 carbon atoms. Because fecal recovery of n-alkanes improves with increasing chain length, C_{32}:C_{33} is usually selected. However, C_{32} and C_{33} are not always present in sufficient quantities to be utilized as internal markers.

The objectives of our study were to identify which n-alkane was present in sufficient quantities to be used as an internal marker, compare its effectiveness as an internal marker with indigestible acid detergent fiber (IADF) for five forages differing in maturity, compare effects of different drying methods on n-alkane extraction from feces and determine if n-alkane disappearance observed during passage through the gastrointestinal tract occurs in the rumen.

Procedure

Five yearling steers (avg body weight = 925 lb) were housed individually in 10 ft x 10 ft pens for five independent digestion trials using mixed grasses from subirrigated meadow (meadow), meadow regrowth and native Sandhills range (range), mature mixed grass hay from meadow and alfalfa hay. The meadow, range, and meadow regrowth trials were conducted in 1995 using immature grasses, beginning June 1, July 1 and

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July 15, respectively, at the Gudmundsen Sandhills Research Laboratory located near Whitman, Nebraska. The meadow hay and alfalfa hay trials were conducted in September 1996 at the West Central Research and Extension Center, North Platte, Nebraska.

Each trial consisted of a 10-day diet adaptation period followed by five days of total fecal collection. Each forage was limit-fed twice daily at 2 percent of body weight with forage from meadow, meadow regrowth and range harvested 0.5 hours before feeding. Feed samples were collected and frozen before each feeding, refusals were collected and frozen before the morning feeding and feces were collected and frozen twice daily.

To examine C_{31} disappearance in the rumen, forages collected during digestion trials were initially subjected to *in vitro* fermentation in which samples weighing 1.8 g were measured into three *in vitro* tubes in 0.6 g increments, inoculated with a mixture of rumen fluid: McDougall's buffer, and were incubated in a 39°C water bath for either 48 or 96 hours. The contents from the three tubes were filtered through filter paper and the residues were combined to form a single sample and saved for later n-alkane analysis.

Laboratory Analyses

Diets, refusals and feces for each trial were freeze dried and ground to pass through a 1-mm screen using a Wiley mill. Samples were composited across days on a per animal basis. Feces from the meadow hay and alfalfa hay trials were subsampled and either freeze dried or dried in a forced air oven (60°C) to compare the effect of drying method on n-alkane extraction. Laboratory analysis included DM, organic matter and IADF. N-alkane analysis was conducted by placing either 1 g of feces or 2 g of forage in a 75-ml tube with 0.6 ml of a 1,000 ppm solution of C_{32} in n-hexane as an internal standard. Ten ml of alcoholic potassium hydroxide were added to each sample and the samples were placed in a water bath at 90°C for 4.5 hours. Upon removal from the water bath, 7 ml of n-hexane and 2 ml of water were added to each tube. After shaking vigorously, samples were centrifuged and the n-hexane layer was transferred to a prepared column for solid phase separation. The column was prepared by first placing 2 g of silicic acid per column in a 110°C oven to activate the silicic acid. Then the silicic acid was suspended in solution using 10 ml of n-hexane and placed in an extraction column. The extract eluted from the column was evaporated, and reconstituted with 2 ml of n-hexane and placed into a glass vial for later analysis using gas chromatography. Ten samples from the meadow, meadow regrowth and range trials selected randomly were reconstituted with 2 ml of a triacontane standard (0.3 mg per ml of n-hexane) to determine the recovery rate of dotriacontane during the extraction procedure.

Results

N-alkane C_{31} was selected as the internal marker to estimate DMD. Although n-alkane recovery increases with increasing chain length, C_{33} was not detected in the freshly harvested forages, most likely due to a lack of column sensitivity where amounts less than 20 mg/kg are not detectable. Even though the efficiency of the procedure averaged 82 percent, this procedure uses both the natural and synthetic n-alkane ratios as a correction factor when efficiencies are less than 100 percent.

For all forages, the digestibility estimates (Table 1) calculated using n-alkane ratio were lower ($P < .01$) than *in vivo* DMD. Comparison of digestibilities estimated using the n-alkane ratios and IADF ratios showed that n-alkane ratios predicted higher DMD for meadow ($P < .01$), meadow regrowth ($P = .06$), and alfalfa hay ($P = .06$), and lower DMD for meadow hay ($P < .02$). Forage digestibilities for native range using n-alkane ratio tended to be higher ($P = .14$) than IADF ratio values. Although C_{31} consistently underestimated the *in vivo* digestibilities for all forages examined, it offered an improvement over digestibilities estimated with IADF for the freshly harvested forages. *In vitro* dry matter disappearance appeared to produce estimates of digestibility comparable to the n-alkane ratio method for immature, freshly harvested forages and higher estimates for alfalfa and meadow hay.

Replacing freeze-drying of fecal samples with oven-drying would decrease the amount of drying time and increase the number of samples handled. While C_{31} amounts in feces from steers fed meadow hay were not affected ($P > 0.10$) by drying method, oven-drying reduced ($P < 0.01$) the amount of C_{31} recovered from the feces of steers on an alfalfa hay diet by 20 percent. During oven-drying, the high temperatures may subject C_{31} to either degradation or chemical reactions that make complete extraction difficult. Since C_{31} concentrations vary with drying methods in both forage and feces, it is recommended that samples should be freeze-dried for n-alkane analysis.

In vitro fermentation was used to determine if C_{31} was degraded in the rumen. Filter paper rated to retain particles greater than 25μm was used to isolate the residue because earlier work indicated n-alkanes are associated with the particulate phase of digesta. The amount of C_{31} found in residues collected by filtration after a 48-hour *in vitro* fermentation

Table 1. Apparent dry matter digestibility.

	In vivo DMD	C_{31}	P<[a]	IADF	P<[b]	*In vitro* DMD
	% DM					
Meadow	67.5	62.9	.004	57.2	.0013	61.5
Range	70.5	61.8	.004	58.3	.14	58.3
Regrowth	70.7	57.5	.002	51.0	.06	57.8
Alfalfa	60.2	50.0	.01	43.8	.06	58.2
Meadow Hay	55.1	36.2	.0001	42.6	.01	47.1

[a] Comparison between *in vitro* digestibility and digestibility predicted using C_{31}.
[b] Comparison between digestibilities predicted using C_{31} and IADF.

period decreased ($P < .001$) over 100 mg/kg. However, samples incubated for 96 hours produced residues similar ($P = .78$) to those produced after 48 hours of incubation. Initial examination of the results indicated that C_{31} is highly degraded in the rumen. However, the digestibility trials with the same forages showed an average total tract recovery of 76.3 percent. Recovery of C_{31} in the residue left after *in vitro* fermentation was approximately 60 percentage units lower. While *in vitro* fermentation could degrade C_{31} to a greater extent than gastrointestinal passage, large differences are unlikely. Because the C_{31} amounts found in the residues remained unchanged between 48-hour and 96- hour incubation times, we propose low recovery was due to association of the marker with the liquid phase which was lost during filtration rather than degradation. Further examination is recommended to determine the digesta phase with which C_{31} associates during gastrointestinal passage.

Locating the site of n-alkane disappearance is important when evaluating its use as a potential internal marker. If disappearance is isolated to the lower tract, the marker may be used to estimate forage dry matter digestibility in the rumen. Because n-alkanes need to be intimately associated with the material they are marking to be reliable as internal markers, it is important the digesta phase association of n-alkanes be determined.

We concluded that: 1) in grazing situations where internal markers need to be used and dosing of synthetic n-alkanes is not practical, naturally occurring n-alkanes may be a better alternative to IADF for immature forages even though digestibility will still be underestimated; 2) C_{31} recovery was not consistent across forages; and 3) freeze-drying should be used to dry fecal samples for n-alkane analysis.

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